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Section B.—Biological Sciences.

Studies in the Mechanism of Enzyme Action. I.—*Rôle of the Reaction of the Medium in Fixing the Optimum Temperature of a Ferment.*

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(Communicated by Prof. B. Moore, F.R.S. Received July 24, 1920.)

Previously, it has been shown for the enzyme *maltase*—enzyme requiring an acid medium in which to act to best advantage—that increase in the acidity or hydrogen ion concentration of the medium in which the enzyme acts, beyond the optimum acidity, leads to a fall of optimum temperature.* The mechanism of this temperature effect appears clearly to be due to a certain disablement of the enzyme, brought about by the presence of excess of acid, for the fall of optimum temperature which occurs is rigorously proportional to the decrease of enzymic activity, estimated at the optimum temperature point; which decrease of activity is itself a function of the degree of acidity of the medium in excess of that necessary to produce optimum activation.* Being in this way a disablement effect, the question arises whether, by adding to the quantity of enzyme in action, the lowering of optimum temperature which takes place can be controlled. To answer that question, the experiments described in the present paper were undertaken.

For the investigation, the enzyme used is the *maltase* of *Aspergillus oryzæ*, the same preparation being employed as studied by us in two previous communications,† a specially active specimen of takadiastase, purified by repeated solution in water and reprecipitation by alcohol.

Fig. 1, based on one of the foregoing communications,* indicates the general lowering of the temperature range and corresponding fall of optimum temperature, for the *maltase* of the preparation in question, when

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* Arthur Compton, 'Roy. Soc. Proc.,' B, vol. 88, pp. 408–417 (1915).

† Arthur Compton, 'Roy. Soc. Proc.,' B, vol. 88, pp. 258–263 (1914), and *loc. cit.*

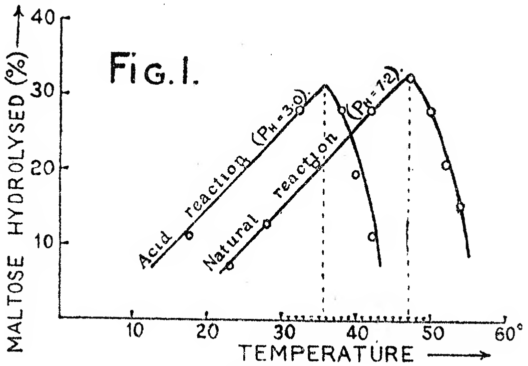

Fig. 1.

0·6 cm.^{3} M/100 H^2SO^4 is added to a reaction mixture of total volume 5 cm.^{3}, containing in solution 3 mgrm. of the enzyme preparation (enzyme concentration, 6×10^{-4} mgrm. per cm.^{3}). This addition of acid, as indicated on the diagram, corresponds to a H^+ concentration of $10^{-3 \cdot 0}$, the H^+ concentration for the natural reaction (*i.e.*, without addition of acid) being $10^{-7 \cdot 2}$. Under these circumstances, the *maltase* of the preparation is seen to undergo a lowering of its optimum temperature from +47° (natural reaction) to +35·5° (above mentioned acid reaction).

In the experiments which follow, we have always operated in presence of this constant addition of acid: 0·6 cm.^{3} H^2SO^4 per 5 cm.^{3} of the reaction mixture, for disabling effect; while the quantities of enzyme present have varied from 3 mgrm. to 7 mgrm. per 5 cm.^{3} total mixture, that is to say, enzyme concentrations varying between 6×10^{-4} and 14×10^{-4} grm. per cm.^{3}. The substrate concentration throughout was M/20, or 18×10^{-3} grm. of hydrated maltose per cm.^{3} of the reaction mixture. It was not practical to study the course of events for superior enzyme concentrations, owing to complete hydrolysis of the substrate being attained, in the conditions of the experiments, and consequent want of definition in the matter of the optimum temperature point.

The practical details of the experiments carried out were as follows: 90 mgrm. of hydrated maltose, 0·6 cm.^{3} M/100 H^2SO^4, and 3·4 cm.^{3} of pure water—prepared from distilled water by redistillation under diminished pressure—were introduced into four series of eight dry, clean, "resistance glass" test-tubes, rendered clean by successive washing in acid, water, and steam. The tubes were then plunged into water thermostats, regulated to the 1/10th of a degree, and, an equilibrium of temperature having been established between their contents and the thermostats, into corresponding tubes of the respective series were introduced in portions of 1 cm.^{3}, solutions of the enzyme, prepared half-hour to one hour previously, containing respectively 3·0 mgrm., 4·75 mgrm., 6·0 mgrm., and 7·0 mgrm. of the enzyme powder per cm.^{3}. The tubes were now closed with selected corks—treated just before use with boiling water from the jet of a wash bottle—and incubated at the temperatures of the thermostats for 16 hours. At the end of this time, the enzyme action was stopped by plunging the tubes for seven minutes into boiling water, the corks having first been withdrawn, and each rapidly washed with 1 cm.^{3} of distilled water, the washings being added to the contents of the corresponding tubes. On being cooled to laboratory temperature, the contents of each tube were diluted to 50 cm.^{3}, and the percentage of maltose hydrolysed, determined by Bertrand's method,* on 20 cm.^{3} of the diluted mixture. The numbers obtained are set out in the accompanying Table.

Temperature at beginning and end of each experiment.	Maltose hydrolysed per cent. with the following enzyme concentrations.			
	6×10^{-4}.	$9{\cdot}5 \times 10^{-4}$.	12×10^{-4}.	14×10^{-4}.
17·0	—	—	21·1	28·2
17·0–17·1	—	16·9		
17·7–17·8	11·4			
22·9–23·2	—	—	—	46·4
24·7–24·9	—	—	40·6	
25·0–24·7	—	28·2		
25·2–25·4	21·1			
32·0	—	—	65·2	
32·1–32·0	—	—	—	82·0
32·2–32·0	—	47·9		
32·2–32·1	28·2			
35·4	—	—	—	94·3
35·5	—	52·5	75·1	
37·0–36·9	—	—	—	92·5
37·8–38·2	28·2			
38·0–38·3	—	—	71·7	
38·3–39·0	—	47·9		
40·0	19·7			
42·0	—	—	54·1	76·8
42·0–42·2	—	29·7		
42·3–42·2	11·4			
45·0	—	—	24·0	49·4
45·2–45·4	—	14·2		
47·6–47·2	—	—	—	24·0

On plotting as ordinates the percentage of maltase hydrolysed, and as abscissæ the corresponding mean temperature, these numbers give the graphical representation of fig. 2.

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* 'Bull. Soc. Chim.,' (3), vol. 35, p. 1285 (1906).

This figure shows that for the acidity in question of the medium addition of more enzyme has merely increased the rate of hydrolysis without changing in any way the optimum temperature, the latter remaining fixed in the neighbourhood of 35·5°, and that no matter what the enzyme concentration. This result was quite unsuspected.

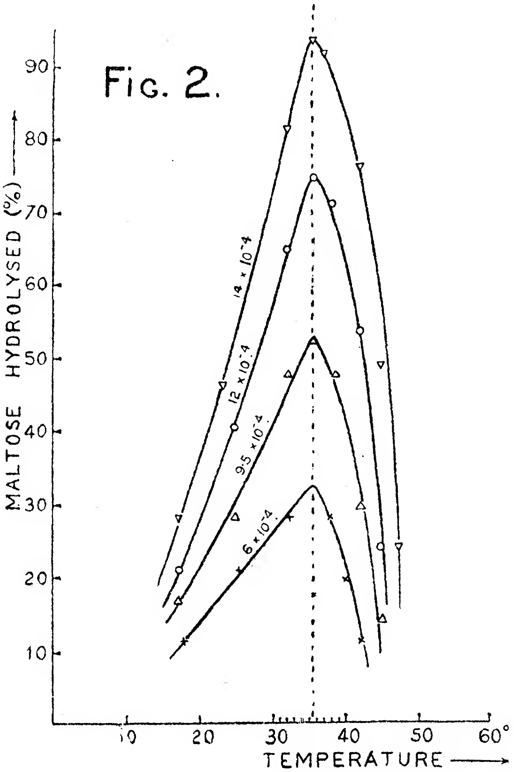

Fig. 2.—Subtrate concentration M/20. Enzyme concentrations 6×10^{-4} to 14×10^{-4} grm. per cm. Reaction of medium—acid.

As, however, it recalls the corresponding course of events for the natural reaction—the enzyme preparation being dissolved in pure water, without the addition of acid as above—fig. 3 is here reproduced for convenience of reference from former work,* carried out in this respect with the same preparation of *maltase.* In the experiment, of which fig. 3 is the graphical *résumé,* the enzyme was employed over a wider range of dilutions than in the foregoing acid experiment: in concentrations varying between 2×10^{-4} grm. and 20×10^{-4} grm. per cm.^{3}, while the subtrate concentration and the duration of the experiment were the same. Under these circumstances, an optimum temperature of +47° is seen to obtain, and that no matter what the enzyme concentration.

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* Arthur Compton, 'Roy. Soc. Proc.,' B, vol. 88, pp. 258–263 (1914), and *loc. cit.*

Figs. 2 and 3, in the constancy of the optimum temperatures which they respectively record, are very striking and indicate clearly the *rôle* of the chemical reaction, or H^+ concentration, of the medium in fixing the optimum temperature of the ferment.

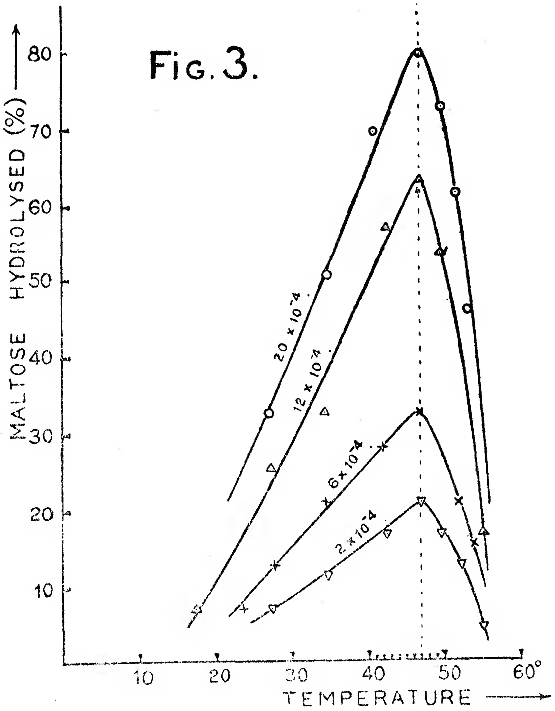

Fig. 3.—Subtrate concentration M/20. Enzyme concentrations 2×10^{-4} to 20×10^{-4} grm. per cm.^{3} Reaction of medium—natural reaction.

Actual measurements were made of the H^+ concentration, by Sorensen's calorimetric method,* for the different enzyme dilutions employed in the two foregoing series of experiments. For the natural reaction series (fig. 3), the H^+ concentrations of the different dilutions were found to be all identical, while the same was found to be true of the acid series (fig. 2), the two distinctive values of P_H thus obtained being respectively as indicated in fig. 1.

The variation, then, of the optimum temperature of an enzyme with the chemical reaction, or H^+ concentration, of the medium, previously established by us,* and its actual fixation for any given H$^+$ concentration, as shown to-day, constitute, as will readily be understood, but different expressions of the same phenomenon, being a consequence the one of the other.

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* 'C. R. Lab. de Carlsberg,' vol. 8, p. 148 (1909).

The bearing of these results on the general law of the independence of the optimum temperature of an enzyme of the concentration of the latter, will not have escaped notice. We see in them but instances of the working of this law, which they throw a new light upon, in indicating that in its original statement it is stated too briefly,† and now requires amplification. In consequence, re-stated, it becomes: the optimum temperature of any ferment, or ferment function, occurring in a given enzymic preparation is independent of the concentration of the enzyme, the duration of the action and *the chemical reaction—or* H$^+$ *concentration—of the medium* being constant.

The question asked at the beginning of the investigation thus finds itself answered; and, if in a manner different from the limited sense in which it was formulated, still the answer is none the less instructive, in the light which it throws on a general aspect of the mechanism of enzyme action.

The Effect of Certain Dietary Deficiencies on the Suprarenal Glands.

By C. H. Kellaway, Foulerton Student of the Royal Society.

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[Plate 1.]

During recent years McCarrison (1919 *a*, *b*, and 1920) has published a series of important papers dealing with the effect of deficient diets on the various organs of the body. One very striking result which he has described was a great enlargement of the suprarenal glands, with pronounced increase in their content of adrenaline, in pigeons fed on polished rice and suffering from the consequent polyneuritis. McCarrison's experience led him to put forward the tentative suggestion, that the increased content of adrenaline might be significant of increased output of adrenaline during the development of the disease, and that this might account for the occurrence of œdema both in experimental polyneuritis produced in pigeons and in the wet form of human

* Arthur Compton, 'Roy. Soc. Proc.,' B, vol. 88, pp. 408–417 (1915).

† Arthur Compton, 'Roy. Soc. Proc.,' B, 87, pp. 245–254 (1914); 'Ann. Inst. Past.,' vol. 28, pp. 866–878 (1914).